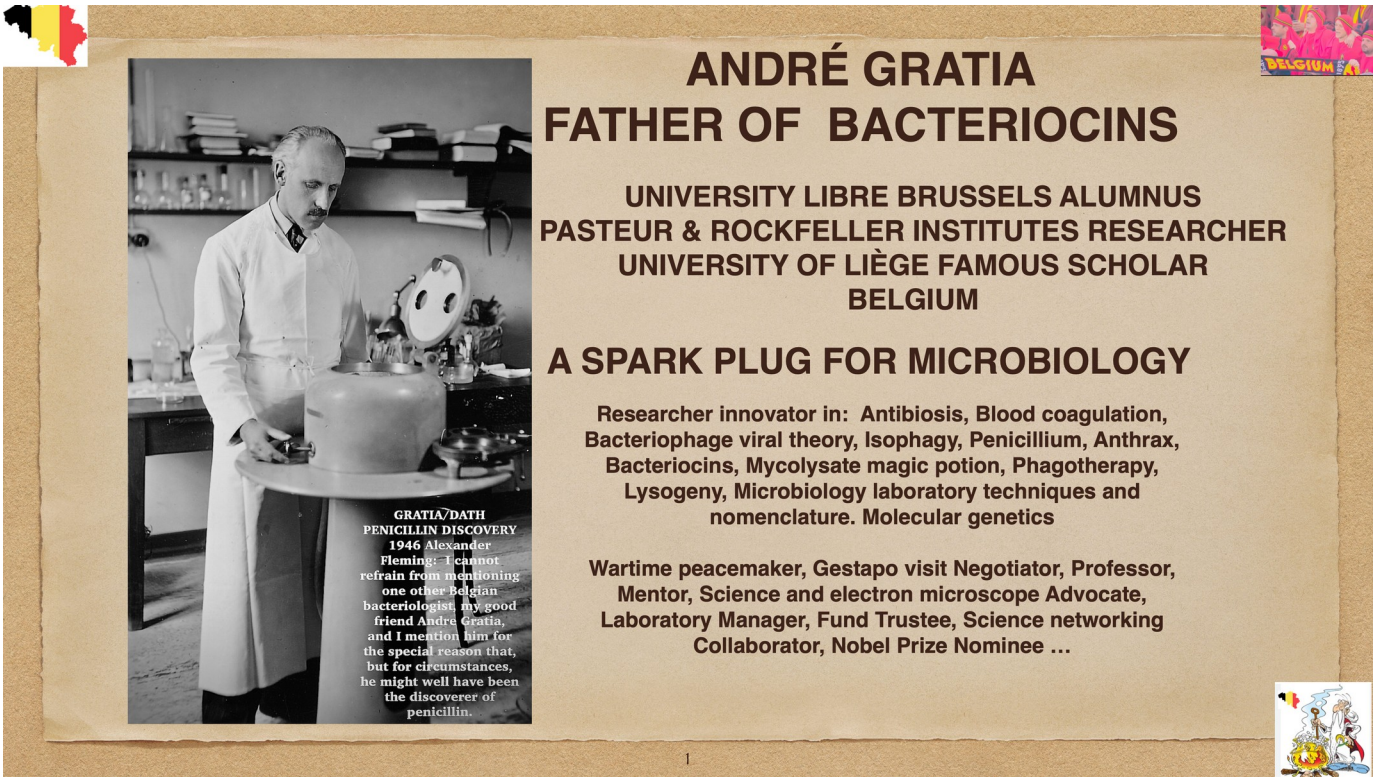
ABSTRACT TO FULL later.

BIOGRAPHY to be done

BELGIUM’S BACTERIOCINS WILL BE BACK!

André Gratia's (**1893-1950**) historic **1925** discovery of **bacteriocins** at the University Libre of Bruxelles is on a comeback! **Bacteriocins are** compounds produced by bacteria affecting other bacteria. They are used in bio-production, fermentation, preservation, sanitation, antibiotics, cloning vectors, and as gene editing plasmids. **On all microbe battlefields as our traditional antibiotics become less**



effective, Gratia's bacteriocins fight against our resistant adapting pathogens, including plant and insect viruses. Gratia was nominated in **1949 for a Nobel Prize in Medicine** with support of his **friend and colleague, Alexander Fleming.**

1946 DIPLOMA CEREMONY, UNIVERSITY OF LIÈGE -- GRATIA TOP RIGHT
Standing: from left to right:
*Prof. Selman **WAKSMAN** (**Rutgers** University), **Nobel Prize** of Medicine **1952** Streptomycin
*Prof. Howard **FLOREY** (**Oxford** University), **Nobel Prize** of Medicine **1945** Penicillin
*Prof Jacques **TREFOUËL** (Institut **Pasteur** Paris) Discovery sulfonamids
*Prof Ernest **CHAIN** (**Oxford** University), **Nobel Prize** of Medicine **1945** Penicillin

*Prof André **GRATIA** (University of **Liège)** Chain expressely requested Gratia at his

side.
Foreground: *Dr Pierre **FRÉDÉRICQ** (University of **Liège**) colicins are plasmid encoded
*Dr Maurice **WELSCH** (University of **Liège)** mass production antibiotics

ANDRÉ GRATIA 1893 - 1950

SPARK PLUG FOR MICROBIOLOGY!

Andre Gratia was born in St. Gilles, Bruxelles in 1893. Motivated and ignited by his **father, Veterinarian Gustave Gratia**, from **Virton**, Belgium, a student of Pasteur who had already proven bovine tuberculosis can be transmitted to humans, and by his **mother**, **Eugénie Solvay.** He voraciously pursues the sciences and donates his entire life to scientific research, creation of new laboratory techniques, methodologies, and the promotion of scientific thoughts and possibilities, innovations and education. **He begins his medical studies at the early age of 17.**

BACTERIA AND BLOOD CLOTTING

WORLD WAR I, 1914 - 1918, SOLDIERS ARE DYING!

Valiantly serving in **WWI** as an **auxiliary doctor,** Gratia earns his *Croix de Guerre* and 8 front line *Chevrons.* Gratia's humanity saddens him painfully when he notices that the wounded who bled most heavily were often hopelessly left to die. Nothing could be done for them. To help these soldiers, in 1919 **Gratia identifies and proves that the Staphylococcus bacteria blood clotting agent**, **Staphylocoagulase, is an enzyme.** This enzyme is still used today in our Staphylococci testing.

Gratia's wartime sadness and memories will remain with him his entire life. He will strive for the peaceful uses of science.

BACTERIA AND VIRUSES — DISTANT COUSINS?

BACTERIOPHAGES — WHAT ARE THEY?

Because of his 1920 research at the **Pasteur Institute Gratia**, now a Doctor of Medicine and Physiology, is invited to continue his endeavours at the **New York Rockefeller Institute for Medical Research under Flexner and Levee.** There he will **serve as model for the composite dashing protagonist for Sinclair Lewis' science thriller *ArrowSmith.*** In **1921 Gratia discover a bacteriophage, a 'phage', a virus,** against the Staphylococcus aureus, a bacteria today very resistant to antibiotics, and Escherichia coli bacteria. This is quite a feat and

a first in the USA! Don't forget that before the electron microscope, bacteriophages were still invisible. Moreover, they were non-filtrable. Nonetheless, **Gratia boldly becomes convinced, and demonstrates experimentally, that Twort's 1915 'glassy' vaccinia lymph' and 1917 D'herelle's bacteriophage are both the same**. Unfortunately at this time, his work with bacteriophages, is unrecognized. Publications were not easily available to be read by all scientists. Gratia´s research is published in medical journals rather than in microbiology journals. It escapes the attention of American microbiology historians. There was no Internet to relay information.

Returning to Belgium Gratia researches and teaches at the **Free University of Brussels Department of Bacteriology. At first a young Gratia dutifully concurs with his well-known supervisor, Laboratory Director Jules Bordet**, 1919 Nobel Prize winner for immunity research, that a bacteriophage might be a sort of external 'fermentation'. This concurrence is clearly shown in the written records of 1922 Glasgow meeting communications. However, **Gratia eventually modifies his interpretation**. He comes to defend and prove in plants and insects **his own view**: **Bacteriophages are eternal infecting particles, viruses** causing diseases, in susceptible bacteria.

THE BACTERIOPHAGE, 'PHAGE', CONTROVERSY IS SETTLED!

BACTERIOPHAGES ARE VIRUSES!

FURTHERMORE, MIGHT PHAGES ALSO BE HELPFUL?

In 1925 Gratia and his colleague Rockefeller colleague, **Paul DeKruif, together publish that bacteriophages are, indeed, viruses.**

Gratia instinctively predicts and demonstrates that some viruses must be good helpful. They could and should be used as therapeutic agents. Today this is known as phagotherapy. However, antibiotics are easier to find and to produce on a large scale. They do not cause other diseases. They do not smell like foul, toilet, excrement because they they aren't

found in our sewage like bacteriophages. Antibiotics, therefore, become the preferred disease curing method in the western world. In contrast, phagotherapy becomes and is still, more popular in eastern Europe. Today, however, phage therapy has started to garner interest momentum in the west among traditional medical practitioners, and especially among clients seeking alternative medical therapies.

By 1936 Gratia has elaborated a simple, clear, useful for phagotherapy, **numerical classification system for bacteriophages, and viruses**. This system is still used worldwide today, without, unfortunately, having Gratia's name attached to it.

BACTERIA VERSUS BACTERIA WAR ZONES!

ATTESTING FOR BROTHERLY CANNIBALISM! ANTIBIOSIS!

Starting in **1924 Gratia**, the perpetual inveterate researcher, as a lucky sideline to his work on phages, **observes with Bernice Rhodes** '**Staphyolococcic isophagy**' **, bacteria cannibalism.** Staphylococcus are destroying the cadavers of other Staphylococcus! They are eating each other!

From this accidental fortuitous new observation, **Gratia wonders**: **Do you think all microorganisms inter-destroy each other? We are on to something!** Let's continue to try to prove this!

Believing that this phenomenon and bacteriophagy might have scientific importance, **Gratia with his lady assistant researcher and co-author, Dr. Sarah Dath, notably begin a systematic search for microbes displaying bacteriolytic activity in nature.** By 1926, Gratia will already have published around 20 isophagy observations between live and dead bacteria, including some similar studies with Escherichia coli.

GRATIA AND HIS COLLEAGUES

MEET DR. SARA DATH, MY ASSISTANT, MY CO-AUTHOR.

At this time women science assistant were, as was Dath, always highly educated. Unfortunately, many were still not honored as co-authors. **Gratia's recognition of Dath's contributions demonstrates his *largess d'esprit.*** It also shows Gratia prioritized science research over the social gender norms of the day.

After Gratia left for the University of Liège in 1932, **Sara Dath remained in Bruxelles**. She taught Hygiene and Bacteriology at the Bruxelles **Edith Cavell Nursing School** from 1935 - 1940 until the 'Urgency' of WWII. She was a dedicated teacher who taught classes at a high 'savant' level to her young ladies nursing students.

BACTERIA AND MOLDS CLASH!

"We searched whether other micoorganismes had similar lytic properties"….

Gratia and Dath were mostly concerned with the bacteriolytic effects of the Actinomycetes bacteria, bacteria similar to molds, rather than those of real molds, fungi, such as Penicillium. However, **together,** they **observe the effects of Penicillium on Anthrax at the Bruxelles Pasteur Institute. In 1924**, Gratia and co-author Dath write:

"From a contaminated culture of Anthrax, completely clarified, (killed), we have isolated a variety of green-coloured diffusible Penicillium talcum dissolving the Anthrax bacillus."

Gratia, André and Dath, Sara. Moisissures et microbes bacteriophages. C. R. Soc. Biol. Paris, 1925, 92, 461-462Propriétés bactériolytiques de certaines moisissures

Gratia, André; Dath, Sara1925 • In Comptes Rendus des Séances de la Société de Biologie et de ses Filiales, 91, p. 1442-1443

This observation was made because of the production of a very actively antibacterial diffusible substance. This substance could have been Penicillin itself. Not all Penicillia produce Penicillin. Please note that we are still only in 1924. Lamentably, after this discovery Gratia becomes seriously ill, depressed, saddened. He abandons his laboratory. Returning to work **in 1929, he finds**

the Penicillium strain dead, and forever lost to the long history of Penicillin, including the observations of Ernest Duchesne in 1897.

1924 — MORE BACTERIA AND BACTERIA INTERMINGLINGS!

Gratia and Dath go on to observe more bacteria interactions, the effects of Streptothrix. Gratia notes the death of Staphylococcus by the secretion products, mixtures of antibiotics and bacteriolitic enzymes, of Streptomyces, another bacteria. **In 1924 Gratia and Dath publish their observation on bacteriolyse by the Actinomycete bacteria, the 'Streptothrix', Streptomycete**.

"They realize that the lytic agent was secreted by the bacteria, even without oxygen. In a classic experiment performed in 1925 – 1926, Gratia and his assistants exposed a 2 percent water agar plate containing dead Staphylococcus to the laboratory air. A culture of white Actinomycetes bacteria grew on the plate surrounded by a clear zone of dissolved bacteria. This airborne bacteria contaminant was next demonstrated to attack killed cultures of Pseudomonas aeruginosa, Mycobacterium tuberculosis and Escherichia coli. **Gratia called this lytic agent 'Mycolysate'.**"

This work has certain similarities with Alexander Fleming's discovery of penicillin, in that it involved bacterial lysis by an airborne contaminant; the difference, however, was that Gratia used killed, rather than living, Staphylococci.

The lysis caused by Streptothrix operates through a very active and eminently diffusible agent that one finds again, separate from the bacteria, in the dissolved and filtrated microbial emulsions."

Gratia, A. Dath S. Propriété bactériologiques de certaines moisissures C R Soc Biol. 1924: 91:1442. Gratia A, Dath S De l'action bacteriolytique des streptothrix C R Soc Bill 1925: 92: 1125

1925 — BACTERIA AND BACTERIA COMMUNICATION SIGNALS!

HERE COME THE BACTERIOCINS! ISOANTAGONISM!

In 1925, while searching for a bacteria with helpful anti-bacterial properties, Gratia describes the activity of a secretion, Colicin 'V', between co-cultures of different strains of E. Coli, the first known bacteriocin!

Colicins are category of bacteriocins. They can kill or inhibit the growth of bacteria strains coming from the producing strain, while not affecting the producing strain. They are members of unrelated family of narrow spectrum bacterially produced antibiotics, requiring the adsorption on specific receptors in the bacterial membrane. They have a protein nature. They are produced by plasmids, also called 'carcinogenic factors', extrachromosomal circular DNA molecules.

However, in a later paper of 1925, it should be acknowledged, Gratia and Dath honestly and candidly note and credit that Lieske had already observed the antibiotic properties of Actinomycetes. They add: "We want to mention ourselves the priority of **Lieske** in this regard".

ARE BACTERIOPHAGES AND BACTERIOCINS DIFFERENT?

YES, THE FIRST ARE ALIVE; THE LATER ARE NOT!

Although Gratia soon **establishes parallelisms between bacteriophages and Colicins,** Gratia **also** points out fundamental **differences between them**. He notes that Colicins are, unlike bacteriophages, diffusible through cellophane, remarkably stable to heat, not thermolabile, not transmissible in series, and have no antigenic activity.

But most importantly, Gratia additionally demonstrates that, because bacteriophages are viruses, they are capable of genetic continuity**; they continue to live!** In contrast, Colicins cannot continue to live. Given this point, colicins cannot be be viruses.

MEDICAL MAGIC POTIONS!

SAVING LIVES EFFICIENTLY WITHOUT POISONOUS TOXICITY!

By 1924 Gratia, always looking for medical benefits**,** already **begins to think of the possibility of using this new discovery as medical therapy.** Gratia concocts a bacteriophage his 'Mycolysate' complex, a mixture of Streptomyces secretions and the Staphylococcus degradation products caused by the bacteriolytic action of Streptomyces which at times may have been supplemented by bacteriocins, bacteriophages, and Penicillium. His **'Mycolysate', a magic potion, successfully soon** efficiently, without toxicity, **saves Belgium lives in the during 1920-30s**.

Gratia has a different explanation for the therapeutic effect of his 'Mycolysate' than Alexander Fleming has for the therapeutic effect of his Penicillin. Gratia believes in a vaccination effect. Fleming believes in an antibacterial effect.

Regardless of whatever opinion we hold now about the relative importance of 'Actinomycetin', as it was called later by Maurice Welsch, Gratia's successor at the University of Liège, and about Penicillin, it cannot be denied that '**Gratia's substance' was used clinically long before Penicillin**, **in 1924. Notwithstanding, Penicillin later proved to be more potent and more practical** than Actinomycetin as a chemotherapeutic agent.

And, Gratia's substance was used, not only, as Florey, Penicillin Nobel Prize laureate, asserts, for immunization purposes, but for therapeutic purposes. It saved lives. Additionally, later these **Actinomycetes became the source of** a series of highly therapeutic substances, such as **Streptomycine, Chloromycetine, Aureomycine, Terramucine. Gratia's 'Mycolysate' mixture** will later reveal to be a mixture of antibiotics and bacteriolytic enzymes.

Gratia believes that his Staphylococcal Mycolysate 'antigen' is best used with a bacteriophage. Bacteriophage therapy was in vogue before antibiotic therapy, but when used often, bacteriophages were said to frequently lose their potency, probably due to a lack of living cells. Gratia believes that by injecting a mixture of Mycolysate and bacteriophages, the

potency of the latter is maintained and also increased. This is probably because Gratia inadvertently had some still-living Staphylococcus cells in his Mycolysate to help the bacteriophages.

Welsch later made a detailed study of the lytic activity of Actinomyces albus. It is generally believed that Actinomycetin was identical to Gratia's Mycolysate. Actinomycetin is an enzymatic protein and is regarded as non-toxic.

"It is not easy to draw conclusions on the therapeutic value of Gratia's Mycolysate because it was not used alone. However, it is certain that the mycolysate was non-toxic. **It constituted a valid therapy but based on products from the bacteriology laboratory of Liège which unfortunately ceased to produce them around 1970.** It is unfortunate that this was not taken up by the pharmaceutical industry because it could have been a successful alternative therapy."

1930's — PARALLELS AMONG RESEARCHERS

Alexander Fleming also took an interest in bacteriophages and in Gratia's 'Mycolysate'. and Gratia mentions that Fleming's work 'should be watched. Fleming's notebooks of 1931 contain 22 pages on bacterial antagonism, including the Mycolysates. The following extract illustrates **how Fleming essentially followed Gratia's experimental instruction protocols:**

"Exposed to air plates of water agar (2%) opaque with killed staphylococci, some colonies cleared Staph., these were Streptothrix. This grew slow on water agar luxuriant on killed staph. agar. Have made mycolysates for other microbes. Can easily make one from streptococcus and now trying effect on chronic or recurring staph. infection. Also pneumo., diphtheria. Insignificant action of BCG. All these can be given to Guinea pigs without any accident. Now studying antibody formation with mycolysate. By injection of guinea pig with mycolysate of diph. get a serum effectively neutralising diph. toxin."

Wainwright, Milton, Sheffield, André Gratia (1893-1950): forgotten pioneer of research into antimicrobial agents UK Keywords: A Gratia; antibiotics; mycolysate; penicilin; colicins; bacteriophage; 1Fleming: HFlorey39-42 Journal of Medical Biography Volume 8 February 2000

1932 — MORE BACTERIOCINS, BACTERIOPHAGES DIFFERENCES!

RECEPTOR SITES SPECIFITIES!

In **1932** Gratia continues researching and teaching at the **University of Liège as the Chair of Parasitology and Bacteriology** and as the **Director of the Bacteriology Service in its Provincial Institute**, **founded by Ernest Malvoz**. He continues his work on phages. He shows they have different properties and specificity that are transmitted to their progeny. He also demonstrates that both phages and colicins have their own specific receptors.

By 1945, Pierre Fredericq and Gratia, will start further study of colicins and find them to have multiple types, each, like phages, endowed with specific receptors." Gratia and Fredericq 1946.

LOCAL IMMUNE RESPONSES — ALLERGY STUDIES

Beyond systemic immune responses, Gratia's curiosity extended to local immune responses, such as **allergy to catgut**, the importance of which is well-known by surgeons, and to the **Sanarelli-Schwartzman localized tissue necrotic** allergy response.

BACTERIA AND VIRUSES COEXISTENCE, LIVING TOGETHER!

LYSOGENY — VIRUSES CAN SLEEP UNNOTICED QUIETLY IN CELLS!

Gratia's interest in bacteria variations and their specific phages led to a key discovery in the understanding of lysogeny, sleeping proviruses lingering in host DNA. Gratia demonstrates that a prophage's life can be inactivated and then reactivated, 1936.

"Another 10 years elapse before M. Delbrück, S. E. Luria, A. D. Hershey, G. Doermann, and then S. Benzer isolate and exploit

mutants of the nontemperate T-even phages in mixed-infection experiments. And even then, the phage group of California Tech. Does not believe in lysogeny and ignores the previous contributions of phage researchers interested in lysogeny studies. They believe that lysogeny is only a reflection of the the carrier bacteria cultures' heterogeneous differences in their sensitivity to phages…

Decades later, noncomplementing diploidy are discovered in hybrids of genetically marked strains obtained either by artificial fusion in B. subtilis (Hotchkiss and Gabor 1980; Guillen et al. 1983, 1986) or by "spontaneous zygogenesis" in E. coli (J. P. Gratia 1994). It has been shown in these cases that a prophage present in one parental strain or a plasmid used to transform one parent is subject to repeated inactivation and reactivation during the descent of stable, phenotypically haploid, heterozygotic noncomplementing diploids. Jean-Pierre Gratia, Perspectives, *Genetics Society of America*, October 2000

LABORTORY TECHNIQUES NOVELTIES!

SPIN, SPIN, SPIN, SPIN! FRACKING — MICROBIOLOGY STYLE!

With Paul Manil from the **Agronomic Institute of Gembloux** and other collaborators**, Jean Brachet and Raymond Jeener** from the **University of Bruxelles** closed by the Axis war occupants**, Gratia carries out sedimentation tests on several viruses and biological materials**. His astuteness. manual dexterity, technical ingenuousity, imagination, experimental rigour help him develop a **laboratory techniques** for **concentrating bacteriophage virus** particles up to 10^{13} particles per milliliter, a record never reached since. Gratia also invents what has long been attributed to Albert Claude, **fractional centrifugation**.

As he begins to develop several new techniques in virology using **air-compressed ultracentifugation**, Gratia reaches contentment, as he finally enters his happiest research period.

ELECTRON MICROSCOPES

WE MUST GET ONE!

To run a top research facility and to attract the elite researchers, one must audaciously have a top laboratory. **Gratia** vehemently advocates and **introduces the electron microscope** developed by the German Ruska, to the **University of Liège**. Ruska was the first to photograph the bacteriophages isolated at the University of Liège. This microscope is alas confiscated by the Americans during the war when they enter Belgium toward the end of World War II in 1945.

In Belgium, as late as 1945, Gratia's group observes that nucleases, RNAse and DNAse enzymes had no effect on four tested bacteriophages of various origins Jeener et al. 1945. Therefore, **these scientists remain perplexed and confused on to the existence and significance of nucleic acids in bacteriophages. No DNA? No RNA?**

Not until 1952 could the physical structure of phage particles be studied by electron microscopy, showing that the nucleic acids are protected against nucleases by an outer capsid.

Gratia also compares phages with plant and insect viruses. Gratia's 1936 serological and ultracentrifugation studies of plant and insect viruses confirms that there exist striking analogies between them and bacteriophages. In 1933 Gratia shows that plant infested with different viruses contain different specific antigens.

WHAT IS THE GENETIC MATERIAL OF VIRUSES?

RNA? OR DNA?, OR BOTH?

Gratia uses the silkworm to research what is the **genetic material of viruses.** Gratia and **André Paillot in 1939 show** that silkworm moths uninfected tissues by the polyhedrons DNA virus has **granules antigenically distinct from the viruses formed from infected tissues.**

Thus with Brachet, Jeener and Paul Manil, Gratia identifies the nature of the nucleic acids carrying the genetic

information of various viruses, those of the vaccinia, the tobacco mosaic and the weeds of the silkworm.

Thanks to the leaves of the mulberry tree on which silkworms feed, of the Institut de Physiologie Leon Fredericq, at the University of Liège, with the collaboration of the French specialist in insect viroses, **Paillot**, Gratia isolates Polyhedra and tiny granules by **differential centrifugation**.

In parallel to A. Claude, who was studying the Rous sarcoma RNA virus and had discovered that noninfected cells contained nucleoprotein granules distinct from the viruses formed in infected cells, **Gratia and Paillot in 1939 showed that silkworm moth noninflected by the Polyhedrons DNA virus had granules anti genetically distinct from the viruses formed from infected tissues**.

Moreover, unlike the Rous Sarcoma RNA Virus which contains RNA like the cytoplasmic nucleoproteins contain Claude 1940, **the granules of infected and uninfected silkworm tissue could be distinguished by their RNA content from the DNA-containing virus** granules present only in infected tissues. Gratia et al. 1945

WORLD WAR II, 1939 - 1945, CIVILIAN UNDERGROUND RESISTANCE

OUR LABORATORY IS LOOTED! RUN! NEGOTIATE!

During **WWII** Gratia's research is hindered and diminished. Gratia is again a patriot. The Resistance is honoured that he is among their members. Twice he is visited by the **Axis Gestapo**. His University of Liège laboratory is looted. On one **Gestapo visit**, keeping his focused cool, in fluent German, he enters into farming medical research discussions with his Gestapo pursuers. using his medical and diplomatic skills to save his life.

"A. Paillot is not the only scientist to have suffered in his flesh from the war: Testimony of André GRATIA in his message of condolences to Mrs. PAILLOT (1945).

"... I recently made a communication to the Belgian Academy of Medicine on my latest research on silkworm grasses, which I think would have interested M. Paillot, to whom I was looking forward to tell about my research. Alas, I must be reduced to beginning my reading with a tribute to his memory. This communication was to have been made last year (1944) at the same time, but I had to give it up at the last minute. No doubt this communication saved my life because it took me (to have to go) to Brussels just when the henchmen of the Gestapo invaded my house (in Cureghem, Belgium?) to stop and terrorize the neighborhood. I had only time to disappear in the Maquis (to the bush) while my family was dispersed to the 4 corners of the country and my poor mother died without my being able to see her again. For 6 months we lived under the threat of being caught. Finally the Liberation seemed to have to put an end to our trials when the offensive of Von Runstradt Renewed the threat. Fortunately, it was stopped at the very doors of Liege, not without one of the sinister robots that ravaged our good city drained of its substance (the research samples) the Institute of Bacteriology where we had taken refuge and where we barely escaped the invaders. ... "

RESISTANCE — MICROORGANISMS STYLE! MUTANTS!

Gratia pioneers in this field because he **was the first, before the 1940s,** to demonstrate the **spontaneous origin of bacterial mutants resistant to bacteriophages and colicins. He also isolates bacteriophage mutants.**

"At the 3rd Congress of Microbiology in New York, **1939, Gratia declares, as Darwin might have done: 'Adaptation by passive selection of pre-existing variants is the only fact to be proven beyond any doubt**'. The Luria Delbrück test, proof of 'cause' or 'selection' for a mutation, was still inconclusive by 1950. The decisive evidence, proof, came with Lederberg's sib selection experiment in **1952**, using replica plating Lederberg 1989. It showed once and for all that the **genesis of streptomycin-resistant mutants in E. coli K12 is independent of the**

presence of the antibiotic in the medium." Jean-Pierre Gratia, Perspectives, *Genetics*, October 2000

Already in 1924 Gratia demonstrates, contradicting Bereska in 1921, that that *B. anthracis* is subject to variation. These variations, such as the rough/smooth bacterial variations are of importance for their disease potential to epidemiologists and bacteriologists.

POST WORLD WAR II

WE MUST EXPAND PENICILLIN AND ANTIBIOTIC RESEARCH!

Gratia continues his research, teaching, managing. **A well-loved, praised, popular, frank, communicative, at times humorous, tactful, caring, enthusiastic, driven teacher,** he continuously advocates for science education, research, promotion and new laboratory techniques for the University of Liège and for science. At the end of the war in **1947**, after liberation, the Belgium government wishes to start a theoretical and applied **Research Center for Penicillin and other Antibiotics, CRPA** in Liège**.** Gratia is appointed to organize its creation, with the support of professors **Selman Waksman, Howard Florey, Boris Chain et Jacques Tréfouël**, and to serve as its president.

Having witnessed the horrors of two world wars, Gratia now heartedly hopes for the **peaceful use of science**, convinced like is father that science is more useful for humanity than religion.

In 1961, this centre becomes the National Centre for the Production and Study of Microbial Substances (CNPEM). It is equipped with fermenters and a whole infrastructure required for the research of antibiotic-producing organisms.

MY EFFORTS ACKNOWLEDGED AND REWARDED

1949 MEDICINE NOBEL PRIZE NOMINATION

In 1949 Gratia is nominated for a Medicine Nobel prize, with support from friend and colleague Fleming, for his work on '**bacterial antibiotics and for seeing and analyzing , viruses and cellular content.'**

1950 FATAL EXHAUSTION

NATIONAL HERO

"It is with heavy astonishment that was greeted the news of the unexpected death of Andre Gratia, first in the heart of the University of Liège then in the city of Liège and the whole country of Belgium, and finally in the foreign scientific community.. Alexander Fleming, Roger Herriott, Salvator Luria, and Wendell Stanley…..I cannot overstate everything about Gratia. I would like to again to evoke that Gratia after being the 'Savant', was also the 'Teacher' in front of his students, and the 'Boss' in his laboratory". Maurice Welsch laments

The traumas of the times prove overwhelming. Overworked and exhausted, Gratia passes in Nyon, Switzerland, where he had gone to rest, leaving his wife Marie-Emmanuel Deltenre, widowed at 40, after a happy life with him, his sons Jean-Pierre and Henri orphaned at 16 and 14 respectively, a multitude of distraught friends and admirers, and his daughter Martine, of his first marriage.

2023 FAMOUS SCHOLAR UNIVERSITY OF LIÈGE ACCLAMATION!

Upon Gratia's passing, **Pierre Frédericq** takes over Gratia's work at the University of Liège. **In 2023 André Gratia is honoured by the University of Liège as a Famous Scholar** with the support of **Dr. Vincent Geenen and Philippe Lecrenier.**

STEPPING STONE FOR BACTERIOCIN RESEARCH TODAY

BELGIUM SYNGULON LABORATORIES

Today **Belgium's Syngulon startup Laboratories, thanks Gratia for his bacteriocin discovery** on which they are

founded, as they work closely with universities to develop and improve bacteriocins technologies.

Colicins are ‘go to’ as plasmids cloning vectors, of small size, with few restriction sites, useful for cloning foreign DNA. In 1950, among Gratia’s last publications, is a display of Petri dishes showing the large diversity of new colicinogenic factors, bacteriocins. .

STUDENTS, DON’T GIVE UP THROUGH HARDSHIPS! FOLLOW ME!

Students, Listen! André Gratia and Sara Dath call you to science discovery. What new horizons will you discover today. Ask them! Follow them! Your journey has just started!

« **La Science est une poule aux œufs d'or** qui pondra dans la mesure où elle est nourrie ; si on la laisse mourir de faim, ce sera la ruine » Gratia 1946 University Alumn Foundationi

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GRATIA’S INNOVATIVE MICROBIOLOGY, VIROLOGY, CELLULAR LABORATORY TECHNIQUES:

1**)The microbial agar technique**: The procedure involves suspension of bacteria 10^{7}-10^{8} in an 0.7 percent agar-agar solution for the demonstration of antibiotic or bacteriolytic properties of substances produced by colonies of bacteria or molds. This technique was reported to Selman Waksman, a specialist in actinomycetes and discoverer of several antibiotics produced by certain species of these bacteria, by Maurice Welsch staying in the USA.

2) **The cellophane sheet procedure:** The technique involves affixing a cellophane sheet to the microbial agar containing a sensitive germ and seeded with a culture of an antibiotic germ to assess the degree of diffusion by the pores of the cellophane of the substances studied

3) The Gratia double layer technique for the titration of bacteriophages has been used by all bacteriophage specialists since its publication in 1936.

4) Gratia also developed a technique for demonstrating **the inhibition of the binding of a bacteriophage to membrane receptors** by the specific antiserum of the phage studied (in collaboration with Willy Mutsaars).

5) Gratia was the first to developed the very efficient technique of **ultracentrifugation using compressed air** in the demarcation between virus particles and protein molecules. Gratia works with the famous **Hendriot and Huguenard compressed air ultracentrifugue**.

6) Gratia developed the **fractional centrifugation** technique before its application by other biologists, including Albert Claude. Ebonite tubes are filled with a suspension of virus or protein substances which after centrifugation are frozen and cut into slices whose content is titrated after thawing. The technique makes it possible to show the differences in sedimentation of the centrifuged elements.

7) **The technique of replicas:** This involves affixing a piece of sterile cloth containing nails to a round wooden block, which is applied to a Petri dish containing bacteriophage plaques, and replicated on a soft agar dish containing specific phage-sensitive bacteria. This procedure ensures the large-scale development of phage particles. The agar where the phage grew is collected and centrifuged. The resulting phage particle titer is surprisingly high. A related technique is that of J. Lederberg, twenty years later, to replicate colonies of bacteria on different media, a technique commonly used in bacterial genetics.

8) Gratia also experimented with **electron microscopy** to identify staphylophages, but did not receive credit for this, due to the Second World War. His electron microscope was taken by the Americans, probably to prevent the microscope being taken by other groups. It was the German Ruska who took pictures of his staphylophages.

REWARDS AND DISTINCTIONS Prix Glüge en 1919, Chevalier de l'Ordre de Léopold en 1934, Prix Laborie en 1937, Prix Helme en 1939, Officier de l'Ordre de la Couronne en 1940, Docteur honoris causa à Gand en 1947, Member Royal Academy of Rome, Royal Academy of Belgium.

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“L’oeuvre scientifique d’André Gratia est remarquable par don abondance — elle comporte quelque deux cents publications — autant que par sa qualité”. Maurice Welsch Octobre 1950 In Memoriam: André Gratia

Please enjoy and download the Gratia and Dath story for science classes, newsletters and archives:

https://www.youtube.com/watch?v=zdDj5KBKR9I 2 minutes

https://www.youtube.com/watch?v=Wib0jCcSDDg. 52 minutes

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Professor of Embryology and History of Biomedical Research at the University of Liège, Chief of the Endocrinology Clinic at CHU of Liège

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Tonse Raju's March 13 Nobel Chronicle (p 936) Nobel chronicle: ‘Fleming and Gratia’

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“SirTonse Raju's March 13 Nobel Chronicle (p 936)1 examines the work of Alexander Fleming, Ernst Boris Chain, and Howard Walter Florey. The work of Fleming and Andre Gratia on the bacteriolyse is also important. The two men knew each other well and took part in high-level scientific meetings on this subject.

Andre Gratia (1893–1950) was a pupil of Jules Bordet (Nobel prize winner in 1919). He worked successively for the Free University of Brussels with Bordet, then for the University of Liege in 1932.

In 1924, Gratia and Sara Dath published their observations on the bacteriolyse via a mould, an actinomycete, the “streptothrix” (streptomycete).2 They realised that the lytic agent was secreted by the mould, even without oxygen.3 For many people, Gratia is the father of the antibiotics.

The next year, they observed another lytic agent for an anthrax culture that was lysed by a mould, a variety of Penicillium glaucum.4 Gratia also discovered the mould of a type of penicillium that he used to treat furunculosis. Gratia may well have been the first to observe bacteriolytic effects of various types of penicillium. By coincidence, 3 years later, Fleming made the same observation with P notatum. This observation was later developed by Florey and Chain and led to an important advance in medicine.

When Fleming received his Doctor Honoris Causa from the University of Liege in 1945, it was Gratia who had written and presented the opening speech for the diploma. During the next year, Fleming was interviewed for the Belgian radio and his text was also published. Here is what **Fleming says in this text: “I cannot refrain from mentioning one other Belgian bacteriologist my good friend Andre Gratia, and I mention him for the special reason that, but for circumstance, he might well have been the discoverer of Penicillin.** In 1926 he noticed that a mould apparently destroy and dissolve certain bacteria…The mould which he had might have been Penicillium

notatum and the active substance might have been penicillin but as the culture was not preserved we shall never know.”

Gratia's mentor, Bordet, would probably have told him: “My boy, the problem with you is that you do not baptize your children!”

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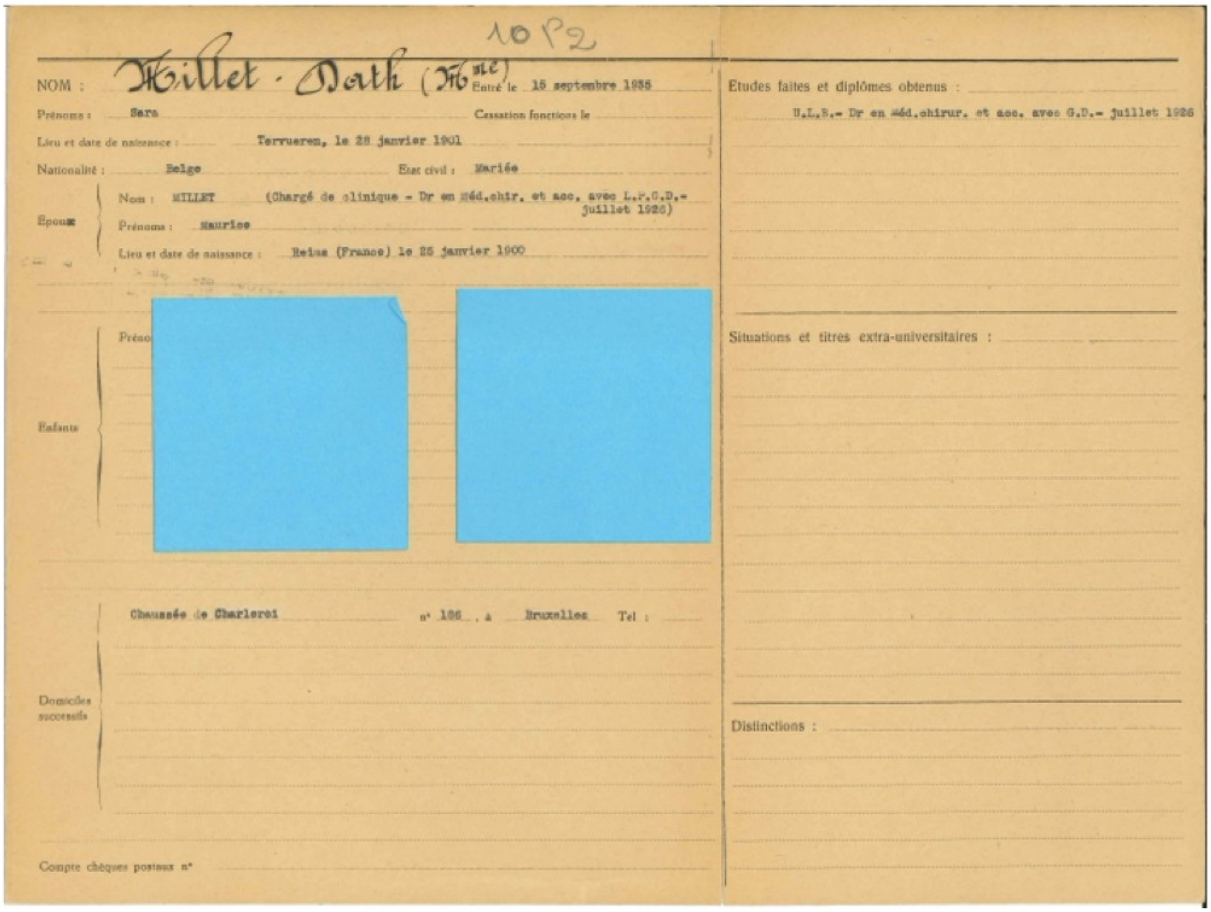

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NOM : Millet - Dath (Mme) Entré le 15 septembre 1935

Prénoms : Sara Cessation fonctions le

Lieu et date de naissance : Terrueren, le 28 janvier 1901

Nationalité : Belge Etat civil : Mariée

Epoux — Nom : MILLET (Chargé de clinique - Dr en Méd.chir. et acc. avec L.P.G.D.- juillet 1926)

Prénoms : Maurice

Lieu et date de naissance : Reims (France) le 25 janvier 1900

Enfants — Préno

Chaussée de Charleroi n° 136 à Bruxelles Tel :

Domiciles successifs

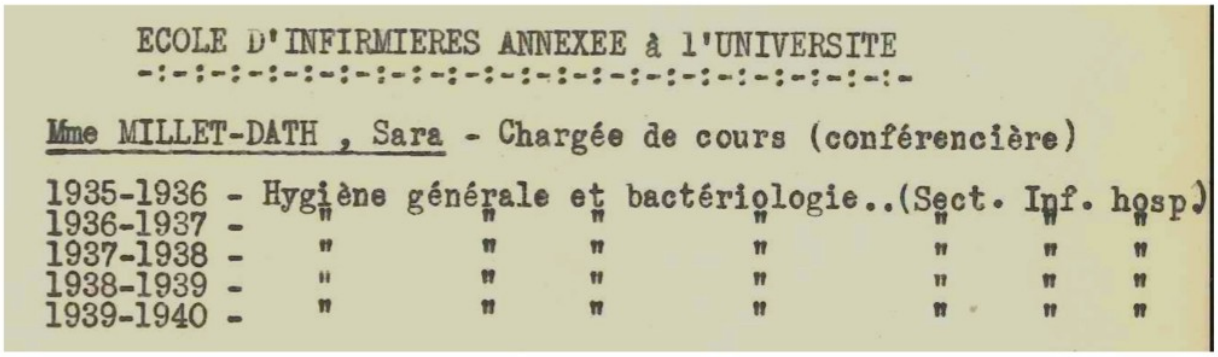
Compte chèques postaux n°

Etudes faites et diplômes obtenus : U.L.B.- Dr en méd.chirur. et acc. avec G.D.- juillet 1926

Situations et titres extra-universitaires :

Distinctions :

RECORD EMPLOYMENT — DR. SARA DATH EDITH CAVELL NURSING SCHOOL



ECOLE D'INFIRMIERES ANNEXEE à l'UNIVERSITE

-:-

Mme MILLET-DATH , Sara - Chargée de cours (conférencière)

1935-1936 - Hygiène générale et bactériologie..(Sect. Inf. hosp.)
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